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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS	:	THOMAS KRAHN ET AL.
SERIAL NO.	:	CONTINUATION OF U.S. SERIAL NO. 09/194,099 FILED ON NOVEMBER 20, 1998
FILED	:	HEREWITH
FOR	:	MASKING BACKGROUND FLUORESCENCE AND LUMINESCENCE IN OPTICAL ANALYSIS OF BIOMEDICAL ASSAYS
ART UNIT	:	UNASSIGNED
EXAMINER	:	UNASSIGNED

September 28, 2001

Hon. Commissioner of Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

SIR:

Prior to examination, please amend the above-identified application as follows:

IN THE SPECIFICATION:

Insert as the first paragraph of the specification the following new paragraph: -- This application is a continuation of U.S. Serial No. 09/194,099, filed on November 20, 1998, now pending. --

IN THE CLAIMS:

Cancel all of the claims in the application and substitute the following new claims:

6. A method of reducing non-specific background light from a biomedical assay comprising:
contacting said biomedical assay with a masking dye, which reduces non-specific background light, wherein said masking dye is substantially membrane;
wherein said biomedical assay comprises biological cells on a solid support;
wherein said biomedical assay comprises a fluorescent dye, which has membrane permeability; and
wherein the fluorescent dye and the masking dye are in an aqueous solution that contacts said biological cells.

7. The method of claim 6, wherein the masking dye has an absorption spectra that overlaps with the emission and/or excitation spectrum of the fluorescent dye.

8. The method of claim 6, wherein the fluorescent dye is a membrane potential-sensitive dye.

9. The method of claim 7, wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce light emitted from said aqueous solution by at least 10% compared to the light emitted of said aqueous solution in the absence of said masking dye.

10. The method of claim 7, wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce light emitted from said aqueous solution by at least 70% compared to the light emitted of said aqueous solution in the absence of said masking dye.

11. The method of claim 6, wherein said masking dye is non-toxic to said biological cell.

12. The method of claim 6, wherein said masking dye is present in said aqueous solution at a concentration of at least 5 mM.

13. The method of claim 6, wherein said masking dye is Brilliant Black.

14. A method of reducing non-specific background light from a biomedical assay comprising:
contacting said biomedical assay with a masking dye,
wherein said biomedical assay comprises biological cells,
wherein said biomedical assay comprises a fluorescent dye, which has membrane permeability and which is in contact with said biological cells;
wherein said masking dye is present in said biomedical assay at a concentration, that reduces non-specific background light from said biomedical assay by at least 30% compared to the light emission from said biomedical assay in the absence of said masking dye,
wherein said masking dye is substantially membrane impermeant;
wherein said masking dye does not specifically bind to said biological cell.

15. A method of claim 14, wherein said non-specific background light is derived from solution fluorescence.

16. A method of claim 14, wherein said masking dye has an absorption spectra that overlaps with the emission and/or excitation spectrum of said fluorescent dye.

17. A kit for performing a biomedical assay, comprising:
a) a fluorescent dye; and
b) a masking dye,
wherein said masking dye is substantially impermeant to the membrane of a biological cell,

wherein said masking dye has an absorption spectrum that overlaps with the emission and/or excitation spectrum of said fluorescence dye,

wherein said masking dye does not specifically bind to said membrane of said biological cell, and

wherein said masking dye is present in a solution at an amount sufficient to reduce non-specific background light emitted from said solution by at least 10% compared to the non-specific background light emitted from said solution in the absence of said photon reducing agent.

18. The kit of claim 17, wherein said masking dye is present in said solution at an amount sufficient to reduce non-specific background light from said solution by at least 30% compared to the non-specific background light emitted from said solution in the absence of said masking dye.

19. The kit of claim 17, wherein said masking dye is present in said solution at an amount sufficient to reduce non-specific background light from said solution by at least 50% compared to the non-specific background light emitted from said solution in the absence of said masking dye.

20. The kit of claim 17, wherein said masking dye is present in said solution at an amount sufficient to reduce non-specific background light from said solution by at least 70% compared to the non-specific background light emitted from said solution in the absence of said masking dye.

21. The kit of claim 17, wherein said fluorescent dye is permeant to the membrane of said biological cell and detects a voltage across the membrane of said biological cell.

22. The kit of claim 17, wherein said fluorescent dye comprises Brilliant Black.

23. The kit of claim 17, wherein said masking dye improves the optical signal-to-noise-ratio by at least 300% compared to the optical signal-to-noise-ratio of said biomedical assay in the absence of said masking dye.

24. A composition of matter, comprising:

a) a biological cell in contact with a solid surface, wherein said biological cell is in contact with a fluorescent dye, wherein said fluorescent dye is permeant to the membrane of said biological cell,

b) an aqueous solution with a masking dye, wherein said aqueous solution is in contact with the membrane of said biological cell,

wherein said masking dye is substantially impermeant to said membrane of said biological cell,

wherein said masking dye has an absorption spectrum that overlaps with the emission and/or excitation spectrum of said fluorescent dye, and

wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce non-specific background light emitted from said solution by at least 10% compared to non-specific background light emitted from said aqueous solution in the absence of said masking dye.

25. The composition of matter of claim 24, wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce light emitted from said fluorescent dye in said aqueous solution by at least 30% compared to the light emitted from said fluorescent dye in said aqueous solution in the absence of said masking dye.

26. The composition of matter of claim 24, wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce light emitted from said fluorescent dye in

said aqueous solution by at least 70% compared to the light emitted from said fluorescent dye in said aqueous solution in the absence of said masking dye.

27. The composition of matter of claim 24, wherein said masking dye has an absorption spectrum that overlaps with the emission and/or excitation spectrum of said fluorescent dye.

28. The composition of matter of claim 24, wherein said composition further includes a microtitre plate and said biological cell is a member of a plurality of biological cells in a well of said microtitre plate.

29. The composition of matter of claim 24, wherein said composition further comprises a system to launch light of a predetermined wavelength through said solid surface the biological cells are in contact with, wherein said predetermined wavelength is an excitation wavelength for said fluorescent dye.

30. The composition of matter of claim 24, wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce non-specific background light emission from said aqueous solution by at least 70% compared to non-specific background light emission in said aqueous solution in the absence of said masking dye.

31. The composition of matter of claim 24, wherein said masking dye is present in said aqueous solution at a concentration of at least 5 mM.

32. A method for identifying a chemical with a biological activity, comprising:
a) contacting a biomedical assay with a test sample,
b) contacting said biomedical assay with a masking dye,
wherein said biomedical assay comprises biological cells in contact with a solid surface,

wherein said biomedical assay comprises a fluorescent dye, which has membrane permeability and which is in contact with said biological cells and which directly or indirectly monitors the activity of said cells,

wherein said masking dye is in an aqueous solution, that contacts the outer surface of the cells, and

wherein said masking dye has an absorption spectra that overlaps with the emission and/or excitation spectrum of said fluorescent dye,

wherein said masking dye is substantially impermeant to the membrane of said cells,

wherein said masking dye does not specifically bind to said cells,

wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce non-specific background light from said aqueous solution by at least 10% compared to the light emitted of said aqueous solution in the absence of said masking dye,

a) detecting an optical signal from said fluorescence dye,

b) comparing the optical signal from said fluorescent dye to a separate control signal from a control batch.

33. A method of claim 32, wherein said fluorescence dye is a potential sensitive dye and wherein the activity of said cells comprises membrane potential changes.

34. A method of claim 32, wherein membrane potential changes below 5 mV can be detected.

35. A medical compound identified by a method comprising the steps of:

a) contacting a biomedical assay with a test sample,

b) contacting said biomedical assay with a masking dye,

wherein said biomedical assay comprises biological cells in contact with a solid surface,

wherein said biomedical assay comprises a fluorescent dye, which has membrane permeability and which is in contact with said biological cells and that directly or indirectly monitors the activity of said cells,

wherein said masking dye is in an aqueous solution, that contacts the outer surface of the cell, and

wherein said masking dye has an absorption spectra that overlaps with the emission and/or excitation spectrum of said fluorescent dye,

wherein said masking dye is substantially impermeant to the membrane of said cells,

c) detecting an optical signal from said fluorescence dye.

36. A method of reducing non-specific background light from a biomedical assay comprising:

contacting said biomedical assay with a masking dye, which reduces non-specific background light;

wherein said biomedical assay comprises a receptor layer which is specific for a fluorescent or luminescent ligand in contact with a solid support;

wherein said biomedical assay comprises fluorescent or luminescent ligands, which are in contact with said receptor layer; and

wherein the masking dye is in an aqueous solution, that contacts the receptor layer.

37. A composition of matter, comprising:

a) a receptor layer in contact with a solid surface, wherein said receptor is specific for a fluorescent or luminescent ligand, and

b) an aqueous solution with a masking dye and with fluorescent or luminescent ligands, wherein said aqueous solution is in contact with said receptor layer,

wherein said masking dye has an absorption spectrum that overlaps with the emission and/or excitation spectrum of said fluorescent ligands,

and

wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce non-specific background light emitted from said solution by at least 10% compared to non-specific background light emitted from said aqueous solution in the absence of said masking dye.

38. The method according to claim 6, wherein the masking dye comprises dye pigments or inorganic finely divided particles.

39. The method according to claim 6, wherein said masking dye has a solubility in water of >2 mg/ml.

40. The method according to claim 6, wherein said masking dye is non-toxic to said biological cell.

41. The method according to claim 6, wherein said masking dye includes Brilliant Black.

42. The method according to claim 6, wherein the fluorescent dye comprises $Dibac_4(3)$.

REMARKS

This application is a continuation of U.S. Serial No. 09/194,099 (hereinafter "the parent application"), which is still pending. A Notice of Allowance was received in connection with the parent application, and thereafter Applicants became aware of the last four U.S. patent documents listed on Sheet 2 of the Form PTO 1449 attached to the accompanying information disclosure statement. A request has been filed to withdraw the parent application from issue to gain consideration of these references, which Applicants believe disclose and/or claim subject matter similar to that presently claimed here and in the parent application. This continuation is being filed to get additional coverage for methods, compositions and kits supported by and corresponding to the method claims being prosecuted in the parent application. For the Examiner's convenience, a mark-up of the newly added claims is attached, which mark-up shows the support for the newly added claims.

Early and favorable action is earnestly solicited.

Respectfully submitted,

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MARK-UP SHOWING SUPPORT FOR NEW CLAIMS:

6. A method of reducing non-specific background light **(p.1, l.16; p.2, l.28)** from a biomedical assay **(p.1,l.2; p.1, l.14)** comprising:

contacting said biomedical assay with a masking dye, which reduces non-specific background light **(p.2, l.29-p.3, l.4),** wherein said masking dye is substantially membrane impermeant **(p.8, l.17);**

wherein said biomedical assay comprises biological cells **(p.1, l.14,15; p.2, l.12 "cellular assay")** on a solid support **(Fig. 1,2...);**

wherein said biomedical assay comprises a fluorescent dye, which has membrane permeability **(p.8, l.28,29 "dispersion fluorescent dye", p. 10, l.9-13)**; and

wherein the fluorescent dye and the masking dye are in an aqueous solution **(p.4, l.20-22),** that contacts said biological cells **(p.1, l.5).**

7. The method of claim 6, wherein the masking dye has an absorption spectra that overlaps with the emission and/or excitation spectrum of the fluorescent dye. **(p.2, l.31-p.3, l.2; p.8, l.19,20)**

8. The method of claim 6, wherein the fluorescent dye is a membrane potential-sensitive dye. **(p.2, l.11-14)**

9. The method of claim 7, wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce light emitted from said aqueous solution by at least 10% compared to the light emitted of said aqueous solution in the absence of said masking dye. **(Fig. 4: without masking dye grey value at U is 70; Fig. 5: with masking dye grey value at U is 15; that is a reduction of light emission of 71%; at least 10% covers 71%, because if you can reach 71% reduction you can reach any reduction below by just adding less masking dye.)**

10. The method of claim 7, wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce light emitted from said aqueous solution by at least 70% compared to the light emitted of said aqueous solution in the absence of said masking dye. **(see claim 9)**

11. The method of claim 6, wherein said masking dye is non-toxic to said biological cell. **(p.4, l.23; p.8, l.22)**

12. The method of claim 6, wherein said masking dye is present in said aqueous solution at a concentration of at least 5 mM. **(p.9, l.5-8, with higher concentration, the masking will work even better).**

13. The method of claim 6, wherein said masking dye is Brilliant Black. **(p.8, l. 30,31)**

14. A method of reducing non-specific background light from a biomedical assay comprising: **(see claim 6)**
contacting said biomedical assay with a masking dye, **(see claim 6)**
wherein said biomedical assay comprises biological cells, **(see claim 6)**
wherein said biomedical assay comprises a fluorescent dye, which has membrane permeability and which is in contact with said biological cells; **(see claim 6)**
wherein said masking dye is present in said biomedical assay at a concentration, that reduces non-specific background light from said biomedical assay by at least 30% compared to the light emission from said biomedical assay in the absence of said masking dye, **(see claim 9)**
wherein said masking dye is substantially membrane impermeant; **(see claim 6)**
wherein said masking dye does not specifically bind to said biological cell. **(p.8, l.28,29 "dispersion fluorescent dye", p. 10, l.9-13, dispersion dyes do not specifically bind to biological cells).**

15. A method of claim 14, wherein said non-specific background light is derived from solution fluorescence. **(p.1, l. 23-25)**

16. A method of claim 14, wherein said masking dye has an absorption spectra that overlaps with the emission and/or excitation spectrum of said fluorescent dye. **(see claim 2)**

17. A kit for performing a biomedical assay, comprising **(p. 2, l.31-p.3, l.2 "in addition to the fluoerescent dye, a further dye is added...",):**

a) a fluorescent dye; and

b) a masking dye,

wherein said masking dye is substantially impermeant to the membrane of a biological cell, **(see claim 6)**

wherein said masking dye has an absorption spectrum that overlaps with the emission and/or excitation spectrum of said fluorescence dye, **(see claim 7)**

wherein said masking dye does not specifically bind to said membrane of said biological cell, and **(see claim 14)**

wherein said masking dye is present in a solution at an amount sufficient to reduce non-specific background light emitted from said solution by at least 10% compared to the non-specific background light emitted from said solution in the absence of said photon reducing agent. **(see claim 9)**

18. The kit of claim 17, wherein said masking dye is present in said solution at an amount sufficient to reduce non-specific background light from said solution by at least 30% compared to the non-specific background light emitted from said solution in the absence of said masking dye. **(see claim 9)**

19. The kit of claim 17, wherein said masking dye is present in said solution at an amount sufficient to reduce non-specific background light from said solution by at least 50%

compared to the non-specific background light emitted from said solution in the absence of said masking dye. **(see claim 9)**

20. The kit of claim 17, wherein said masking dye is present in said solution at an amount sufficient to reduce non-specific background light from said solution by at least 70% compared to the non-specific background light emitted from said solution in the absence of said masking dye. **(see claim 9)**

21. The kit of claim 17, wherein said fluorescent dye is ~~im~~permeant to the membrane of said biological cell **(see claim 6)** and detects a voltage across the membrane of said biological cell. **(p. 2, l. 11-17)**

22. The kit of claim 17, wherein said fluorescent dye comprises Brilliant Black. **(see claim 13)**

23. The kit of claim 17, wherein said masking dye improves the optical signal-to-noise-ratio by at least 300% compared to the optical signal-to-noise-ratio of said biomedical assay in the absence of said masking dye. **(9, l. 16,17 "factor of 4=400%" is covered by at least 300%)**

24. A composition of matter, comprising:

a) a biological cell in contact with a solid surface, wherein said biological cell is in contact with a fluorescent dye, **(Fig. 1,2, p.7, l. 12-16)** wherein said fluorescent dye is permeant to the membrane of said biological cell, **(see claim 6)**

b) an aqueous solution with a masking dye, wherein said aqueous solution is in contact with the membrane of said biological cell, **(see claim 6)**

wherein said masking dye is substantially impermeant to said membrane of said biological cell, **(see claim 6)**

wherein said masking dye has an absorption spectrum that overlaps with the emission and/or excitation spectrum of said fluorescent dye, **(see claim 7)** and

wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce non-specific background light emitted from said solution by at least 10% compared to non-specific background light emitted from said aqueous solution in the absence of said masking dye. **(see claim 9)**

25. The composition of matter of claim 24, wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce light emitted from said fluorescent dye in said aqueous solution by at least 30% compared to the light emitted from said fluorescent dye in said aqueous solution in the absence of said masking dye. **(see claim 9)**

26. The composition of matter of claim 24, wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce light emitted from said fluorescent dye in said aqueous solution by at least 70% compared to the light emitted from said fluorescent dye in said aqueous solution in the absence of said masking dye. **(see claim 9)**

27. The composition of matter of claim 24, wherein said masking dye has an absorption spectrum that overlaps with the emission and/or excitation spectrum of said fluorescent dye. **(see claim 7)**

28. The composition of matter of claim 24, wherein said composition further includes a microtitre plate and said biological cell is a member of a plurality of biological cells in a well of said microtitre plate. **(p. 5, l. 3; p.5, l.29)**

29. The composition of matter of claim 24, wherein said composition further comprises a system to launch light of a predetermined wavelength through said solid surface the biological cells are in contact with, wherein said predetermined wavelength is an excitation wavelength for said fluorescent dye. **(Fig. 1, p. 7, l. 23, 24)**

30. The composition of matter of claim 24, wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce non-specific background light emission from said aqueous solution by at least 70% compared to non-specific background light emission in said aqueous solution in the absence of said masking dye. **(see claim 9)**

31. The composition of matter of claim 24, wherein said masking dye is present in said aqueous solution at a concentration of at least 5 mM. **(see claim 12)**

32. A method for identifying a chemical with a biological activity **(p. 2, l. 17, 18; p. 5, l. 26-p. 6, l. 1)**, this is what screening does), comprising:

a) contacting a biomedical assay with a test sample, **(p. 2, l. 17,18 "high sample throughput")**

b) contacting said biomedical assay with a masking dye, **(see claim 6)**

wherein said biomedical assay comprises biological cells in contact with a solid surface, **(see claim 24)**

wherein said biomedical assay comprises a fluorescent dye, which has membrane permeability and which is in contact with said biological cells **(see claim 1)** and which directly or indirectly monitors the activity of said cells, **(p. 5, l. 27, 28)**

wherein said masking dye is in an aqueous solution, that contacts the outer surface of the cells, and **(see claim 6 and Fig. 1)**

wherein said masking dye has an absorption spectra that overlaps with the emission and/or excitation spectrum of said fluorescent dye, **(see claim 7)**

wherein said masking dye is substantially impermeant to the membrane of said cells, **(see claim 6)**

wherein said masking dye does not specifically bind to said cells, **(see claim 14)**

wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce non-specific background light from said aqueous solution by at least 10% compared to the light emitted of said aqueous solution in the absence of said masking dye, **(see claim 9)**

c) detecting an optical signal from said fluorescence dye, **(p. 3, l. 2-4)**

d) comparing the optical signal from said fluorescent dye to a separate control signal from a control batch. **(p. 5, l. 11-15)**

33. A method of claim 32, wherein said fluorescence dye is a potential sensitive dye and wherein the activity of said cells comprises membrane potential changes.**(p.2, l. 11-14)**

34. A method of claim 32, wherein membrane potential changes below 5 mV can be detected. **(p. 2, l. 14-16)**

35. A medical compound identified by a method comprising the steps of:

a) contacting a biomedical assay with a test sample, **(see claim 6)**

b) contacting said biomedical assay with a masking dye, **(see claim 6)**

wherein said biomedical assay comprises biological cells in contact with a solid surface, **(see claim 24)**

wherein said biomedical assay comprises a fluorescent dye, which has membrane permeability and which is in contact with said biological cells **(see claim 6)** and that directly or indirectly monitors the activity of said cells, **(p. 5, l. 27, 28)**

wherein said masking dye is in an aqueous solution, that contacts the outer surface of the cell, and **(see claim 6)**

wherein said masking dye has an absorption spectra that overlaps with the emission and/or excitation spectrum of said fluorescent dye, **(see claim 7)**

wherein said masking dye is substantially impermeant to the membrane of said cells, **(see claim 6)**

c) detecting an optical signal from said fluorescence dye.**(see claim 32)**

36. A method of reducing non-specific background light from a biomedical assay comprising:

contacting said biomedical assay with a masking dye, which reduces non-specific background light; **(p. 2, l. 28-p. 3, l.4)**

wherein said biomedical assay comprises a receptor layer which is specific for a fluorescent or luminescent ligand in contact with a solid support;

wherein said biomedical assay comprises fluorescent or luminescent ligands, which are in contact with said receptor layer; and

wherein the masking dye is in an aqueous solution, that contacts the receptor layer.

(p. 3, l. 21-p.4, l.2; Fig. 10)

37. A composition of matter, comprising:

a) a receptor layer in contact with a solid surface, wherein said receptor is specific for a fluorescent or luminescent ligand, and

b) an aqueous solution with a masking dye and with fluorescent or luminescent ligands, wherein said aqueous solution is in contact with said receptor layer,

wherein said masking dye has an absorption spectrum that overlaps with the emission and/or excitation spectrum of said fluorescent ligands,

(p. 3, l. 21-p.4, l.2; Fig. 10)

and

wherein said masking dye is present in said aqueous solution at a concentration sufficient to reduce non-specific background light emitted from said solution by at least 10% compared to non-specific background light emitted from said aqueous solution in the absence of said masking dye. **(analog to claim 9)**

38. The method according to claim 6, wherein the masking dye comprises dye pigments or inorganic finely devided particles. **(p.5, l. 23)**

39. The method according to claim 6, wherein said masking dye has a solubility in water of >2 mg/ml. **(p. 8, l. 24)**

40. The method according to claim 6, wherein said masking dye is non-toxic to said biological cell. **(see claim 11)**

41. The method according to claim 6, wherein said masking dye includes Brilliant Black. **(see claim 13).**

42. The method according to claim 6, wherein the fluorescent dye comprises $Dibac_4(3)$. **(see p. 8, l. 28,29).**